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(54) ENANTIOMERICALLY PURE BETA-D-DIOXOLANE-NUCLEOSIDES

ENANTIOMERISCH REINE BETA-D-DIOXOLAN-NUKLEOSIDE

NUCLEOSIDES ENANTIOMERIQUEMENT PURS DE BETA-D-DIOXOLANE

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(56) References cited:
EP-A- 0 337 713
WO-A-90/12023
US-A- 5 041 449
US-A- 5 204 466
EP-A- 0 515 156
WO-A-92/15308
US-A- 5 047 407

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Description

Background of the Invention

[0001] This invention is in the area of organic compounds with antiviral activity, and the use of said compounds in the treatment of viral diseases that includes administering an effective amount of one or more of the described compounds.

[0002] A number of 2',3'-dideoxynucleosides have been found to be potent antiviral agents against human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS). AZT (3'-azido-2'-deoxythymidine, Mitsuya, H.; Broder, *S. Proc. Natl. Acad. Sci. U.S.A.*, 1986 83, 1911) was the first compound approved by the U.S. Food and Drug Administration for the treatment of patients with AIDS or AIDS-related complex. Other synthetic nucleosides have now either been approved or are undergoing various stages of clinical trials, including 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC) (see Yarchoan, R. et. al., *Science,* 1989, 245, 412), and 2'-fluoro-arabinofuranosyl-2'-3'-dideoxycytidine (Martin, T.A., et al., *J. Med. Chem.*, 1990, 33, 2137; Watanabe, K.A., et al., *J. Med. Chem.*, 1990, 33, 2145; Sterzycki, R.Z., et al., *J. Med. Chem.*, 1990 33, 2150).

[0003] After cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides may be incorporated into a growing strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group.

[0004] The stereochemistry of nucleoside derivatives play an important role in their biological activity. The C1' position of the ribose in the nucleoside (the carbon bound to the nitrogen of the heterocyclic base) is a chiral center because the carbon is attached to four different moieties. Likewise, there is an optically active center at C4' of the nucleoside (the ring carbon bound to the hydroxymethyl group that is phosphorylated in nucleotides). In the naturally occurring nucleosides, the base attached to the C1' and the hydroxymethyl group attached to the C4' atom are in the β-configuration (above the plane of the sugar). The corresponding non-naturally occurring α-isomers (in which the moieties are below the plane of the sugar) are rarely biologically active, and are typically toxic.

[0005] An analysis of the solid-state conformations of six active and two inactive anti-HIV nucleoside agents was recently performed to attempt to correlate the presence or absence of certain stereochemical features with high HIV activity. Van Roey, P., et al., *J. Am. Chem. Soc., 1988*, 110, 2277; and Van Roey, P., et al., *Proc. Natl. Acad. Sci. U.S. A.*, 1989, 86, 3929.

[0006] There has been recent interest in the synthesis of nucleoside derivatives in which the 3'-carbon of the nucleoside has been replaced with a heteroatom. Norbeck, D.W., et al., in *Tet. Lett.*, 1989, 30, 6263, reported the synthesis of (±)-1-[(2β,4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (referred to below as (±)-dioxolane-T, see Figure 1), that results in a racemic mixture of diastereomers about the C4' atom. The product is a derivative of 3'-deoxythymidine in which the C3' atom has been replaced with an O3' atom. The product was synthesized in five steps from benzyloxyacetaldehyde dimethylacetal and (±)-methyl glycerate to produce a 79% yield of the 1:1 diastereomeric mixture. The X-ray crystallographic analysis of the product revealed that the dioxolane ring adopts the $^3T_4$ conformation commonly observed in ribonucleosides, with the O3' atom in the endo position. Norbeck reported that the racemic mixture of dioxolane-T exhibits an anti-HIV activity of 20 μM in ATH8 cells, and attributed the low efficacy against the virus to an effect of the endo conformation of the O3' atom. Tetrahedron Letters 30 (46), 6246, (1989).

[0007] European Patent Application Publication No. 0 337 713 and U.S. Patent No. 5,041,449, assigned to IAF BioChem International, Inc., disclose that a generic formula of 2-substituted-4-substituted-1,3-dioxolanes exhibit antiviral activity.

[0008] Belleau, et al., in the Fifth International Conf. on AIDS, Montreal, Canada June 4-9, 1990, paper No. T.C.O. 1., reported a method of synthesis of cytidine nucleosides that contain oxygen or sulfur in the 3'-position. The dioxolane ring was prepared by the condensation of RCO_2CH_2CHO with glycerin. As with the Norbeck synthesis, the Belleau synthesis results in a racemic mixture of diastereoisomers about the C4' carbon of the nucleoside. Belleau reported that the sulfur analog, referred to as NGBP-21 or (±) BCH-189 (see Figure 1), has anti-HIV activity.

[0009] European Patent Application No. 92300056.6 to Belleau disclosed the use of BCH-189 for the treatment of hepatitis B virus (HBV).

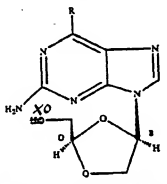
[0010] U.S. Patent No. 5,047,407 and European Patent Application Publication No. O 382 526, also assigned to IAF Biochem International, Inc. disclose that a generic formula of 2-substituted-5-substituted-1,3-oxathiolane nucleosides have antiviral activity.

[0011] WO 92/10497 (EP 562 009) discloses an asymmetric synthesis for the production of β-(D)-dioxolane nucleosides, including (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine, which comprises preparing the dioxolane ring from 1,6-anhydromannose.

[0012] It is another object of the present invention to provide enantiomerically pure dioxolane nucleosides with significant anti-HIV activity.

Summary of the Invention

[0013] The use for the treatment of humans infected with HIV that includes administering an HIV treatment amount of an enantiomerically pure β-D-dioxolanyl purine nucleoside of the formula:



wherein R is Cl, NH_2, or H, or a pharmaceutically acceptable salt or derivative of the compound, optionally in a pharmaceutically acceptable carrier or diluent. The compound wherein R is chloro is specifically referred to as (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine. The compound wherein R is hydroxy is (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine. The compound wherein R is amino is (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine. The compound wherein R is hydrogen is (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine.

[0014] The specifically disclosed β-D-dioxolane nucleosides, or their pharmaceutically acceptable derivatives or salts or pharmaceutically acceptable formulations containing these compounds are useful in the treatment of HIV infections and other related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpurea and opportunistic infections. In addition, these compounds or formulations can be used prophylactically to retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

[0015] In another embodiment, the invention includes the use for the treatment of humans infected with HIV that includes administering an HIV treatment amount of a prodrug of the specifically disclosed enantiomerically pure β-D-dioxolanyl purine nucleosides. A prodrug, as used herein, refers to a pharmaceutically acceptable derivative of the specifically disclosed nucleoside, that is converted into the nucleoside on administration in vivo. Nonlimiting examples are pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and the 5' and N^6 acylated or alkylated derivatives of the active compound (alternatively referred to as "physiologically or pharmaceutically acceptable derivatives"). In one embodiment, the acyl group is a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic C_1-C_{20} alkyl alkoxyalkyl including methoxymethyl; aralkyl including benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy; a dicarboxylic acid such as succinic acid; sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl; and the mono, di and triphosphate esters.

[0016] As used herein, the term alkyl specifically includes but is not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, cyclopentyl, and cyclohexyl. As used herein, the term acyl specifically includes but is not limited to acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate, propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic. Modifications of the active compound, specifically at the N^6 and 5'-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

[0017] The enantiomerically pure β-D-dioxolanyl purine nucleoside can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. The nucleoside or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt can be converted into the parent nucleoside, for example, by hydrolysis.

[0018] The invention as disclosed also includes an asymmetric process for the preparation of enantiomerically pure β-D-dioxolane-nucleosides. The process involves the initial preparation of (2R,4R)- and (2R,4S)-4-acetoxy-2-(protect-

ed-oxymethyl)-dioxolane from 1,6-anhydromannose, a sugar that contains all of the necessary stereochemistry for the enantiomerically pure final product, including the correct diastereomeric configuration about the 1 position of the sugar (that becomes the 4'-position in the later formed nucleoside).

[0019] The (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane is condensed with a desired heterocyclic base in the presence of $SnCl_4$, other Lewis acid, or trimethylsilyl triflate in an organic solvent such as dichloroethane, acetonitrile, or methylene chloride, to provide the stereochemically pure dioxolane-nucleoside.

[0020] Any desired enantiomerically pure β-D-dioxolane purine or pyrimidine nucleoside can be prepared according to the process disclosed herein. The product can be used as a research tool to study the inhibition of HIV in vitro or can be administered in a pharmaceutical composition to inhibit the growth of HIV in vivo.

Brief Description of the Figures

[0021] Figure 1A is an illustration of the chemical structure of (±)-1-[(2β,4β)-2-(hydroxymethyl)-4-(1,3-thioxolane)] thymine (BCH-189).

[0022] Figure 1B is an illustration of the chemical structure of (±)-1-[(2β,4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (dioxolane-T).

[0023] Figure 2 is an illustration of the method of synthesis of enantiomerically pure β-D-(-)-dioxolane-thymine.

[0024] Figure 3 is an illustration of the method of preparation of a variety of enantiomerically pure β-D-(-)-dioxolanyl purine nucleosides (reagents: (a) TMSOTf, CH_2Cl_2; (b) NH_3, DME; (c) $HSCH_2CH_2OH$, NaOMe; (d) NH_3, EtOH; (e) n-Bu_4NF, THF.)

Detailed Description of the Invention

[0025] As used herein, the term "enantiomerically pure" refers to a nucleoside composition that includes at least 97% of a single enantiomer of that nucleoside.

I. Preparation of Enantiomerically Pure Dioxolane Nucleosides

[0026] In preparing enantiomerically pure dioxolane nucleosides, care should be taken to avoid strong acidic conditions that would cleave the dioxolane ring. Reactions should be performed, if possible, in basic or neutral conditions, and when acidic conditions are necessary, the time of reaction should be minimized.

A. Preparation of Enantiomerically Pure β-D-Dioxolane-Nucleosides

[0027] The key starting material for the synthesis of enantiomerically pure β-D-dioxolane-nucleosides is 1,6-anhydromannose (compound 1, Figure 2). This sugar contains all of the necessary stereochemistry for the enantiomerically pure final product (see for example, compound 11, Figure 2), including the correct diastereomeric configuration about the 1 position of the sugar (that becomes the 4'-position in the later formed nucleoside). 1,6-Anhydromannose can be prepared according to procedures described in Knauf, A.E.; Hann, R.M.; Hudson, C.S. *J. Am. Chem. Soc.*, 1941, 63, 1447; and Zottola, M.A.; Alonso, R.; Vite, G.D.; Fraser-Reid, B. *J. Org. Chem.*, 1989, 54, 6123. Prior syntheses of dioxolane nucleosides have used racemic mixtures of starting materials for the preparation of the ribose moiety. When the syntheses begin with a racemic mixture of reagents, undesirable racemic mixtures of enantiomeric nucleoside products have been produced. The mixtures are very difficult to separate and significantly increase the cost of the final product. Further, the inclusion of non-naturally occurring isomers increases the toxicity of the product.

[0028] The 1,6-anhydromannose is converted to its isopropylidene derivative with dimethoxypropane and p-toluenesulfonic acid, which, without isolation, is benzoylated in the 4-position to compound 2 (see Figure 2). An acyl group can also be used to protect the 4-position. The isopropylidene group of compound 2 is then removed by a catalytic amount of an acid such as sulfuric acid, hydrochloric acid, formic acid, trifluoroacetic acid, sulfamic acid, in 60% aqueous dioxane or other suitable organic solvent at a temperature range of approximately 0 to 50°C to give (-)-1,6-anhydro-4-0-benzoyl-β-D-mannopyranose in high yield as a white solid.

[0029] In the next step, the glycol of (-)-1,6-anhydro-4-0-benzoyl-β-D-mannopyranose is oxidatively cleaved by treatment with $NaIO_4$ in H_2O/EtOH (1:1) for one hour at approximately room temperature to produce to the corresponding dialdehyde. Lead tetraacetate can also be used as the oxidizing reagent for this reaction. The dialdehyde is immediately reduced in situ with any suitable reducing agent, including $NaBH_4$, diisobutylaluminum hydride (DIBAL-H), lithium borohydride ($LiBH_4$), or sodium bis(2-methoxyethoxy)-aluminum hydride (Red-Al), at approximately room temperature or below. Under the conditions of reaction, compound 4 isomerizes by benzoyl migration from a secondary to a primary position to produce (-)-(2R,4R)-4-(2-benzoxy-1-hydroxyethyl)-2-(hydroxymethyl)-dioxolane (compound 5, Figure 2).

[0030] The 2-position of the dioxolane is then protected with a suitable oxygen protecting group, for example, a

trisubstituted silyl group such as trimethylsilyl, dimethylhexylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, trityl, alkyl group, acyl groups such as acetyl, propionyl, benzoyl, p-NO_2 benzoyl, or toluyl, methylsulfonyl, or p-toluylsulfonyl. A preferred protecting group is t-butyldiphenylsilyl. After protecting the 2-position of the dioxolane, the benzoyl group is removed from the 2-hydroxyethyl-position with a strong base such as sodium methoxide or ammonia in methanol at approximately 0 to 50°C to produce (-)-{2R,4R)-2-(protected-O-methyl)-4-(1,2-dihydroxyethyl)-dioxolane (compound 6, Figure 2) in high yield.

[0031] In the next step, the 1,2-dihydroxyethyl group in the 4-position of the dioxolane is converted to a carboxylic acid with an oxidizing agent such as $NaIO_4/RuO_2$, or lead tetraacetate, at approximately 0 to 50°C to produce (+)-(2R, 4R)-2-(protected-oxymethyl)-4-carboxyldioxolane (see compound 7, Figure 2).

[0032] A modified Hunsdiecker reaction (Dhavale, D.; et al., *Tetrahedron Lett.*, 1988, 29, 6163) is then carried out in ethyl acetate with $Pb(OAc)_4$ to convert (+)-(2R,4R)-2-(protected-oxymethyl)-4-carboxyldioxolane to the corresponding key intermediates (2R,4R)-and (2R,4S)-4-acetoxy-2-(protected-oxymethyl) dioxolane (see compound 8, Figure 2) in good yield.

B. Condensation of a Heterocyclic Base with the Dioxolane Derivative

[0033] In the next step of this reaction scheme, the enantiomerically pure dioxolane prepared as described in Section A. is condensed with a protected base in the presence of trimethylsilyl triflate (trimethylsilyl trifluoromethanesulfonate) or a Lewis acid in a dry organic solvent.

[0034] Any aromatic compound, and in particular a purine or pyrimidine, containing a nitrogen that is capable of reaction with a center of electron deficiency can be used in the condensation reaction. Purine bases include but are not limited to adenine, hypoxanthine, 2,6-diaminopurine, 6amino-2-chloropurine, 2-aminopurine, N^6-alkylpurines, N^6-benzylpurine, N^6-halopurine, and guanine. Pyrimidine bases include but are not limited to thymine, cytosine, 6-azapyrimidine, 2-mercaptopyrimidine, and uracil. Functional oxygen and nitrogen groups on the heterocyclic base should be protected before condensation with the sugar if undesired side reactions occur during the synthetic procedure. Protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, t-butyldimethylsilyl, and t-butyldiphenylsilyl, tritylmethyl, alkyl groups, acyl groups such as acetyl and propionyl, methylsulfonyl, and p-toluylsulfonyl.

[0035] Friedel-Crafts catalysts (Lewis acids) that can be used in the condensation reaction include $SnCl_4$, $ZnCl_4$, $TiCl_4$, $AlCl_3$, $FeCl_3$, BF_3-diethylether, and BCl_3. These catalysts require anhydrous conditions because the presence of water reduces their activity. The catalysts are also inactivated in the presence of organic solvents with active hydrogens, such as alcohols and organic acids. The catalysts are typically used in solvents such as carbon disulfide, methylene chloride, nitromethane, 1,2-dichloroethane, nitrobenzene, tetrachloroethane, chlorobenzene, benzene, toluene, dimethylformamide, tetrahydrofuran, dioxane, or acetonitrile. Anhydrous aluminum chloride is not soluble in carbon disulfide. Niedballa, et al., J. Org. Chem. 39, 25 (1974). The preferred catalyst is $SnCl_4$. The preferred solvent is 1,2-dichloroethane. Trimethylsilyl triflate can be used under the same conditions described above for the Friedel-Crafts catalysts. The reaction proceeds at a temperature range of from -10°C to 200°C. The choice of catalyst for condensation will affect the final product ratio of a to B nucleoside product. For example, condensation of the intermediates (2R,4R)- and (2R,4S)-4-acetoxy-2-(t-butyldiphenylsilyoxymethyl) dioxolane (compound 8, Figure 2) with silylated thymidine in the presence of trimethylsilyl triflate in CH_2Cl_2 gave a mixture of (-)-1-[(2R,4R)-2-(t-butyldiphenylsilyloxymethyl)-4-dioxolanyl]thymine 9-β (45%) and (+)-1-[(2R,4S)-2-(t-butyldiphenylsilyloxymethyl)-4-dioxolanyl] thymine 10-α (29%). However, the reaction with $SnCl_4$ produced exclusively β-isomer 9 with trace amounts of α-isomer 10 detectable on TLC.

[0036] 2,6-Disubstituted purine derivatives were synthesized by the condensation of acetate 8 with the silylated 6-chloro-2-fluoropurine, which gave a mixture (α/β=1/1.3) of 14 and 13 (Figure 3). The initially formed N^7-isomer was again converted to the N^9-isomer during stirring overnight at room temperature. The analytical sample was obtained from the separation of α,β-mixture to the individual isomers 13 and 14 by a preparative TLC using CH_2Cl_2-acetone (19:1) as the developing solvents. However, for the purpose of preparing the final products 21-24, the mixture of 13 and 14 was treated with NH_3 in DME (Robins, M.J.; Vznanski, B. Nucleic acid related compounds. 34. Non-aqueous Diazotization with tert-Butyl nitrite. Introduction of Fluorine, Chlorine, and Bromine at C-2 of Purine Nucleosides. *Can. J.* Chem. 1981, 2608) to give a mixture of 21-24, which was separated to the individual isomers 15 (24%), 16 (18.6%), 17 (25.8%) and 18 (16%). The guanine 19 and 2,6-diamino 20 derivatives were prepared by the treatment of 15 with 2-mercaptoethanol/NaOMe and ammonia in ethanol, respectively. The free nucleosides 21-26 were obtained upon treatment of the corresponding 5'-silylated nucleosides with *n*-Bu_4NF in good yields. The α-isomers 23 and 24 were also prepared by the similar procedure as the β-isomers.

[0037] In the final step of this method of preparation of enantiomerically pure (-)-B-D-dioxolane-nucleosides, the 5'-O-position of the nucleoside is deprotected. Desilylation can be carried out with a variety of reagents, including acetic acid, trifluoroacetic acid, hydrogen fluoride, n-tetrabutylammonium fluoride, potassium fluoride and pyridinium HCl. For example, desilylation of compounds 9 and 10 with tetrabutylammonium fluoride gave the desired free nucleosides 11

and **12**, respectively (Figure 2). Acetic acid is preferred for commercial scale use because it is inexpensive. Other reagents for desilylation are known to those skilled in the art. Deacylation is accomplished in acid or base. 5-O-Ethers can be cleaved with BCl_3 or trimethylsilyl iodide.

[0038] The method of preparation of enantiomerically pure β-D-dioxolane-nucleosides is further illustrated in the following working examples. Example 1 sets out in detail a method for the preparation of (2R,4R)- and (2R,4S)-4-acetoxy-2-(t-butyldiphenylsilyoxymethyl)dioxolane (compound **8**, Figure 2). Example 2 sets out the preparation of (-)-1-[(2β,4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine, referred to as (-)-β-D-dioxolane-T. The enumeration of compounds in Example 2 refer to structures set out in Figure 2. Example 3 provides detailed examples for the preparation of a number of enantiomerically pure β-D-dioxolanyl nucleosides, including (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine, (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine, and (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine.

Example 1 Preparation of (2R,4R)- and (2R,4S)-4-Acetoxy-2-(t-butyldiphenylsilyloxymethyl) dioxolane (Compound 8).

(-)-1,6-Anhydro-2,3-isopropylidene-4-0-benzoyl-β-D-mannopyranose

[0039] 1,6-anhydro-β-D-mannopyranose (compound **1**) was mixed with acetone (800 ml) and methanol (300 ml) and stirred for approximately thirty minutes until only a free-flowing solid remained. Dimethoxypropane (300 ml), and p-toluenesulfonic acid (5 g) were then added, and the mixture stirred for 2 hours.

[0040] The reaction mixture was then made basic with triethylamine (pH 8), and filtered to remove the white solid material. The solvents were evaporated, and the residue taken up in ethyl acetate and then crystallized to obtain 4 grams of the 2,3-isopropylidenated product as clear needles.

[0041] To a solution of the 1,6-anhydro-2,3-isopropylidene-β-D-mannopyranose (5.01 g, 0.025 mol) in pyridine (40 ml) was added dropwise benzoyl chloride (3.74 ml, 0.062 mol) at 0°C. The mixture was stirred for 45 minutes at 0°C. Ice was then added to the reaction mixture to remove excess benzoyl chloride. The solvent was evaporated under vacuum and the residue was dissolved in ethyl acetate (200 ml). The organic layer was washed with water, sat. $NaHCO_3$ and brine. The resulting material was dried over anhydrous $MgSO_4$, filtered, and then evaporated to give (-)-1,6-anhydro-2,3-isopropylidene-4-0-benzoyl-β-D-mannopyranose crude product (compound **2**, 8.7 g) as yellowish solid.

(-)-1,6-Anhydro-4-0-benzoyl-β-D-mannopyranose (3).

[0042] To a solution of 1,6-anhydro-4-0-benzoyl-2,3-isopropylidene-β-D-mannopyranose **2** (10.0 g, 32.6 mmole) in 60% aqueous dioxane (820 ml) was added concentrated H_2SO_4 (3.36 ml). The mixture was stirred at 70~80° C for 15 hours, and then cooled in an ice bath, neutralized with $NaHCO_3$ and concentrated until half of the original volume remained. The solution was then extracted with ethyl acetate and the combined organic layers washed with saturated $NaHCO_3$ solution and water, dried, and evaporated to give **3** as a white solid. The solid was crystallized from CH_2Cl_2-n-hexane to yield **3** (7.4 g, 85.3%) as white solid: $[\alpha]^{25}$D-154.7° (C, 0.21 MeOH); ^{1}H NMR (DMSO-d_6): δ 3.56-4.61 (m, 5H, 2,3,5,6-H), 4.82 (d, J=8.1 Hz, 1H, OH D_2O exchangeable), 5.02 (s, 1H, 4-H), 5.09 (d, J=3.7 Hz, 1H, OH, D_2O exchangeable), 5.28 (s, 1H, 1-H), 7.46-8.05 (m, 5H, Ar-H); IR (KBr) 3410, 1710 cm^{-1}; Anal. Calcd for $C_{13}H_{14}O_6$: C, 58.64; H, 5.31. Found: C, 58.51; H, 5.34.

(-)-(2R,4R)-4-(2-Benzoxy-1-hydroxyethyl) -2-(hydroxymethyl)dioxolane (5).

[0043] To a solution of **3** (7.4 g, 27.8 mmole) in 95% ethanol (200 ml) was added a solution of $NaIO_4$ (6.54 g, 30.7 mmole) in water (200 ml). The mixture was stirred at room temperature for 1 hour. After checking to insure the complete conversion of diol to dialdehyde by thin layer chromatography, the reaction mixture was concentrated to the half of the original volume. Methanol (200 ml) was added to the residue and the mixture was cooled to 50°C. Sodium borohydride (4.2 g, 111.0 mmole) was added to the mixture portion-wise for 5 minutes and the mixture was stirred at 50°C for 10 minutes, neutralized with glacial acetic acid and concentrated to yield crude **3** as yellow oil. The oil was purified by column chromatography over silica gel to yield pure **3** as colorless oil, that was crystallized from diethyl ether/n-hexane to yield **5** (6.12 g, 82%) as white solid: $[\alpha]^{25}$D - 18.5° (C 0.20, methanol); ^{1}H NMR (DMSO-d_6): δ 3.47 (dd, J=5.9, 3.7 Hz, 2H, CH_2OH), 3.72-4.14 (m, 4H, 4, 5-H and CHOH), 4.27-4.95 (m, 2H, CH_2OBz), 4.81-4.95 (m, 2-H and pri OH), 5.43 (d, J=5.5 Hz, 1H, sec OH, D_2O exchangeable), 7.43-8.09 (m, 5H, Ar-H); Anal. Calcd for $C_{13}H_{16}O_6$: C, 58.19; H, 6.02. Found: C, 58.09; H, 6.01.

(-)-(2R,4R)-4-(2-Benzoxy-1-hydroxyethyl)-2-(t-butyldiphenylsilyloxy-methyl)-dioxolane.

[0044] To a solution of 3 (2.8 g, 10.4 mmole) and imidazole (2.04 g, 30.0 mmole) in dimethylformamide (40 ml) was added t-butyldiphenylsilyl chloride (3 ml, 11.5 mmole). The mixture was stirred at room temperature for 2 hours. The reaction mixture was evaporated to yield a yellow oil, that was purified by column chromatography over silica gel to yield 4 (4.48 g, 85%) as a colorless oil; $[\alpha]^{25}D$ - 14.2° (C 0.26, methanol); ^1H NMR (DMSO-d_6): δ 1.00 (s, 9H, t-Bu), 3.68-3.87 (m, 3H, CH_2OTBDPS and CHOH), 3.98-4.16 (m, 3H, 4,5-H), 4.20-4.55 (m, 2H, CH_2OBz), 5.07 (t, J=3.3 Hz, 1H, 2-H), 5.47 (d, J-5.7 Hz, 1H, OH, D_2O exchangeable), 7.40-8.33 (m, 1OH, Ar-H); Anal. Calcd for $C_{29}H_{34}O_6Si$: C, 68.73; H, 6.79. Found: C, 68.86; H, 6.83.

(-)-(2R,4R)-2-(t-Butyldiphenylsilyloxymethyl)-4-(1,2-dihydroxyethyl)-dioxolane (6).

[0045] To a solution of (-)-(2R,4R)-4-(2-benzoxy-1-hydroxyethyl)-2-(t-butyldiphenylsilyloxy-methyl)-dioxolane (2.52 g, 5.0 mmole) in methanol (40 ml) was added a 0.078 M solution of sodium methoxide (7.3 ml) in methanol. The mixture stirred at room temperature for two hours. The mixture was neutralized with acetic acid and concentrated. The residue was then portioned between ethyl acetate and water, and the aqueous layer extracted with ethyl acetate. The combined organic layers were washed with a saturated $NaHCO_3$ solution and then water, and then dried, evaporated, and purified by column chromatography over silica gel to yield 6 (1.9 g, 95%) as colorless oil: $[\alpha]^{25}D$-2° (C 0.25, MeOH); ^1H NMR (DMSO-d_6) δ 1.00 (s, 9H, t-Bu), 3.40-3.52 (m, 3H, CH_2OH and CHOH), 3.64 (d, J=3.7 Hx, 2H, CH_2OTBDPS), 3.82-3.95 (m, 3H, 4,5-H), 4.49 (t, J-5.3 Hz, 1H, pri OH, D_2O exchangeable), 4.82 (d, J=5.1 Hz, 1H, sec OH, D_2O exchangeable), 5.01 (t, J-3.7 Hz, 1H, 2-H), 7.36-7.71 (m, 10H, Ar-H); Anal. Calcd for $C_{22}H_{33}H_{30}O_5Si$: C, 65.63; H, 7.53. Found: C, 65.72; H, 7.52.

(+)-(2R,4R)-2-(t-Butyldiphenylsilyloxymethyl)-4-carboxyldioxolane (7).

[0046] To a biphasic solution of 6 (1.6 g, 4.0 mmole) in CH_3CN (8 ml), CCl_4 (8 ml) and H_2O (12 ml) was added $NaIO_4$ (3.59 g, 16.8 mmole) and RuO_2 hydrate (8.5 mg). The mixture was vigorously stirred at room temperature for 5 hours. Methylene chloride (40 ml) was added to the mixture. The organic layer was separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, filtered through celite pad and then concentrated to yield crude 7 (1.2 g, 77.4%) as black oil, that was used in the next reaction without further purification. For analytical purposes crude 7 was purified by column chromatography over silica gel to yield 7 as a white foam: $[\alpha]^{25}D$ + 15.7° (C 0.28, MeOH); ^1H NMR (DMSO-d_6) δ 0.99 (s, 9H, t-Bu), 3.43-4.05 (m, 4H, 5-H and CH_2OTBDPS), 4.25 (t, J=6.8 Hz, 1H, 4-H), 5.04 (dd, J=5.1, 3.7 Hz, 1H, 2-H), 7.38-7.72 (m, 10H, Ar-H).

(2R,4R)- and (2R, 4S) -4-Acetoxy-2-(t-butyldiphenylsilyoxymethyl) dioxolane (8).

[0047] To a solution of 7 (0.46 g, 1.14 mmole) in ethyl acetate (10 ml) was added pyridine (0.09 ml, 1.25 mmole) and $Pb(OAc)_4$ (0.66 g, 1.49 mmole). The mixture was stirred at room temperature for 15 hours under N_2, and then filtered through celite pad, and then concentrated and purified by column chromatography over silica gel to yield 8 (0.29 g, 63.5%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.06 and 1.10 (s, 9H, t-Bu), 1.92 and 2.06 (s, 1H, CH_3), 3.71-4.24 (m, 4H, 5-H and CH_2OTBDPS), 5.25 and 5.38 (t, J=4.3 and 3.3 Hz each, 1H, 2-H), 6.27-6.41 (m, 1H, 4-H), 7.20-7.72 (m, 10H, Ar-H); IR (KBr) 3400, 1620 cm^{-1}.

Example 2 Preparation of (-)-1-[(2R,4R)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (11).

(-)-1-[(2R,4R)-2-(t-Butyldiphenylsilyloxymethyl)-4-dioxolanyl]thymine (9) and (+)-1-[(2R,4S)-2-(t-Butyldiphenylsilyloxymethyl)-4-dioxolanyl] thymine (10).

[0048] To a suspension of thymine (0.15 g, 1.2 mmole) in haxamethyldisilazane (10 ml) was added a catalytic amount of $(NH_4)_2SO_4$, and the mixture refluxed for 3 hours. The clear solution obtained was concentrated to yield silylated thymine as a colorless oil. A solution of 8 (0.24 g, 0.6 mmole) in CH_2Cl_2 (5 ml) was added to a solution of silylated thymine in CH_2Cl_2 (5 ml) and the mixture cooled to 5°C. To the cooled mixture was added trimethylsilyl triflate (0.23 ml, 1.2 mmole), and the mixture stirred at room temperature for 1 hour under N_2. A saturated $NaHCO_3$ solution (20 ml) was added to the mixture, and the mixture again stirred at room temperature for 30 minutes. The organic layer was then separated and the aqueous layer extracted with CH_2Cl_2. The combined organic layer was washed with a saturated $NaHCO_3$ solution and water, dried, concentrated and separated by column chromatography over silical gel to yield 9 (0.125 g, 44.6%) as white foam and 10 (0.08 g, 28.6%) as white foam: 9 (β-form); $[\alpha]^{25}D$ - 6.98° (C 0.43, MeOH) ; ^1H NMR ($CDCl_3$) δ 1.08 (s, 9H, t-Bu), 1.67 (s, 3H, CH_3), 3.92 (d, J=3.2 Hz, 2H, CH_2OTBDPS), 4.14 (d, J=4.0 Hz, 2H, 5-H),

5.06 (t, J=3.2 Hz, 1H, 2-H), 6.36 (t, J+4.0 Hz, 1H, 4-H), 7.26-7.75 (m, 10H, Ar-H), 9.51 (bnr s, 1H, H=NH): UV (MeOH) λ_{max} 265.0 (pH 2); 264.4 nm (pH 11); Anal. Calcd for $C_{25}H_{30}O_5N_2Si$: C, 64.34; H, 6.49; N, 6.00. Found C, 64.28; H, 6.51; N, 5.98:

10 (α-form); $[\alpha]^{25}D$ + 11.3° (C 0.23, MeOH); ^1H NMR ($CDCl_3$) δ 1.08 (s, 9H, t-Bu), 1.94 (d, J=1.2 Hz, 3H, CH_3), 3.70 (d, J=3.2 Hz, 2H, $CH_2OTBDPS$), 4.01 (dd, J=9.5, 2.3 Hz, 1H, 5H), 4.35 (dd, J=9.5, 5.3 Hz, 1H, 5-H), 5.55 (t, J=3.2 Hz, 1H, 2-H), 6.32 (dd, J=5.3, 2.3 Hz, 1H, 4-H), 7.17 (d, J=1.2 Hz, 1H, 6'-H), 7.37-7.74 (m, 10H, Ar-H), 9.57 (br s, 1H, NH); UV (MeOH) λ_{max} 265.0; (pH 2); 264.5 nm (pH 11); Anal. Calcd for $C_{25}H_{30}O_5N_2Si$: C, 64.34; H, 6.49; N, 6.00. Found C, 64.23; H, 6.51; N, 5.93.

(-)-1-[(2R,4R)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (11).

[0049] To a solution of **9** (93.3 mg, 0.2 mmole) in tetrahydrofuran (THF) (3 ml) was added a 1.0 M solution of tetra-n-butylammonium fluoride in THF (0.24 ml, 0.24 mmole) and the mixture stirred at room temperature for 1 hour. The mixture was then concentrated and purified by column chromatography over silica gel to yield **11** (42 mg, 92.1%) as white solid: $[\alpha]^{25}D$-18.8° (C o.17, MeOH); ^1H NMR (DMSO-d_6) δ 1.75 (d, J=1.2 Hz, 3H, CH_3), 3.63 (dd, J+6.0, 2.6 Hz, 2H, CH_2OH), 4.03 (dd, J=9.9, 5.5 Hz, 1H, 5-H), 4.22 (dd, J=9.9, 2.0 Hz, 1H, 5-H), 4.90 (t, J=2.6 Hz, 1H, 2-H), 5.16 (t, J-t.0 Hz, 1H, OH), 6.21 (dd, J=5.5, 2.0 Hz, 1H, 4-H), 7.67 (d, J=1.2 Hz, 1H, 6'-H), 11.27 (br s, 1H NH); UV (H_2) λmax 266.0 (∈ 10757); 266.5 (∈ 9894) (pH 2); 266.3 (∈ 8397) (pH 11); Anal. Calcd for $C_9H_{12}O_5N_2$: C, 47.36; H, 5.31; N, 12.28. found: c, 47.28; H, 5.34; N, 12.29.

(+)-1-[(2R,4S)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (12).

[0050] Deprotection of **10** (60 mg, 0.13 mmole) according to same procedure as described above for **11** yielded **12** (26 mg, 87.6%) as a white foam: $[\alpha]^{25}D$ + 10.7° (C 0.15, MeOH); ^1H NMR (DMSO-d_6) δ 1.79 (s, 3H, CH_3), 3.43 (dd, J=6.0, 3.7 Hz, 2H, CH_2OH), 4.02 (dd, J=9.5, 3.3 Hz, 1H, 5-H), 4.28 (dd, J=9.5, 5.6 Hz, 1H, 5-H), 5.00 (t, J=6.0 Hz, 1H, OH), 5.47 (t, J=3.7 Hz, 1H, 2-H), 6.17 (dd, J=5.6, 3.3 Hz, 1H, 4-H), 7.43 (d, J=1.2 Hz, 1H, 6'-H), 11.32 (br s, 1H NH); UV (H_2O) λ_{max} 266.5 (∈ 9454); 266.5 (∈ 9199) (pH 2); 266.3 (∈ 6925) (pH=11); Anal. Calcd for $C_9H_{12}O_5N_2$: C, 47.36; H, 5.31; N, 12.28. found: C, 47.22; H.5.32; N, 12.16.

Example 3 Preparation of Enantiomerically Pure β-D-Dioxolanyl Purine Nucleosides

(2R,4R) and (2R,4S) -9-[[2-[(*tert*-Butyldiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-6-chloro-2-fluoropurine (13 and 14).

[0051] A mixture of 2-fluoro-6-chloropurine (4.05 g, 23.47 mmol) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (940 mL) was refluxed for 2 hours. The resulting solution was concentrated under anhydrous conditions to yield silylated 2-fluoro-6-chloropurine as a white solid. To a cooled (0°C) and stirred solution of silylated 2-fluoro-6-chloropurine (5.69 g, 23.69 mmol)b and compound **8** (7.84 g, 19.57 mmol) in dry methylene chloride (175 mL) was added TMSOTf (4.41 mL, 23.44 mmol). The reaction mixture was warmed to room temperature and stirred for 16 hours, during which time, all the initially formed N_7 condensed product was converted to N_9-isomer. The reaction mixture was quenched with saturated $NaHCO_3$ solution (50 mL) and stirred for an additional 20 minutes at room temperature, evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (200 mL), washed with water and brine, dried (anhydrous Na_2SO_4), filtered and evaporated to give a solid residue, which was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford a mixture of B-anomer **19** and α-anomer **20** (1.3: 1; β/α) as a white crystalline solid (6.30 g, 62.8%). The analytical sample was purified by preparative TLC using CH_2Cl_2-acetone (19:1) as the developing system to give **13** (R_f= 0.5p0) and **14** (R_f = 0.55) for NMR characterization: UV (MeOH) λ_{max} 269.0 nm.

(-)-(2R,4R)-2-Amino-9-[[2-[(*tert*-butyldiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-6-chloropurine (15), (-)-(2R, 4R)-9-[[2-[(*tert*-Butyldiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-2-fluoroadenine (16), (+)-(2R,4S)-2-Amino-9-[[2-[(*tert*-butyldiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-6-chloropurine (17) and (+)-(2R,4S)-9-[[2-[(*tert*-Butyldiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-2-fluoroadenine (18).

[0052] Dry ammonia gas was bubbled into a stirred solution of **13** and **14** (6.25 g, 12.18 mmol) in DME (125 mL) overnight. The solvent was evaporated under reduced pressure and the residue was subjected to chromatographic separation of the four compounds on a silica gel column (20-30% ethyl acetate in CH_2Cl_2). **15** (R_f = 0.35, 1.49 g, 24%): a white crystalline solid. UV (MeOH) λ_{max} 309.5 nm. Anal. ($C_{25}H_{26}ClN_5O_3Si$) C, H, Cl, N. **16** (R_f = 0.21, 1.12 g, 18.6%): colorless needles. UV (MeOH) λ_{max}261.0, 268.0 (sh) nm. Anal. ($C_{25}H_{26}FN_5O_3Si$) C, H, F, N. **17** (R_f = 0.43, 1.60 g,

25.76%): a white crystalline solid. UV (MeOH) λ_{max} 261.0, 269.0 (sh) nm. Anal. ($C_{25}H_{28}FN_5O_3Si$) C, H, F, N. **18** (R_f = 0.12, 0.96 g, 16%), a microcrystalline solid. UV (methanol) λ_{max} 261.0, 269.0 (sh) nm. Anal. ($C_{25}H_{28}FN_5O_3Si$) C, H, F, N.

(-)-(2R,4R)-2-Amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine (15).

[0053] A solution of **15** (0.46 g, 0.91 mmol) in THF (20 mL) was treated with 1 M *n*-Bu_4NF/THF (1.1 mL, 1.1 mmol) to give **21** (R_f = 0.50, 0.21 g, 84%) as a crystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 307.0 nm (ε8,370) (pH7), 307.5 (ε8,590) (pH 2), 307.0 (ε8,800) (pH 11). Anal. ($C_9H_{10}ClN_5O_3$) C, H, Cl, N.

(-)-(2R,4R)-2-Fluoro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (28).

[0054] A solution of **16** (0.56 g, 1.12 mmol) in THF (20 mL) was treated with 1 M *n*-Bu_4NF/THF (1.35 mL, 1.35 mmol) to furnish **22** (0.24 g, 85%) as a white crystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 260.8 nm (ε17,010), 268.5 (sh) nm (ε13,510) (pH 7), 261.0 (ε16,390), 268.5 (sh) (ε 13,300) (pH2), 260.8 (ε 16,700), 268.5 (sh) (ε 13,200) (pH 11). Anal. ($C_9H_{10}FN_5O_3$) C, H, F, N.

(-)-(2R,4R)-9-[(2-Hydroxymethyl)-1,3-dioxolan-4-yl]guanine (25).

[0055] A mixture of **15** (0.29 g, 0.57 mmol), $HSCH_2CH_2OH$ (0.51 mL) and 1.0 M NaOMe/MeOH (11.5 mL) in MeOH (20 mL) was refluxed for 3 hours. The reaction mixture was cooled and neutralized with glacial acetic acid. The solution was evaporated to dryness, and then the residue was triturated with $CHCl_3$, filtered and the filtrate was taken to dryness to give crude compound **19** (0.21 g, 75%), which without further purification was subjected to desilylation according to the same procedure described for **23** to give compound **25** (0.07 g, 61%) as a microcrystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 252.0 (ε8,730) (pH 7), 254.4 (ε12,130), 277.5 (sh) (ε8,070) (pH 2), 264.3 (ε10,800) (pH11). Anal. ($C_9H_{11}N_5O_4$) C, H, N.

(-)-(2R,4R)-2-Amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (26).

[0056] A steel bomb was charged with compound **15** (0.28 g, 0.55 mmol), anhydrous ethanol (20 mL) saturated with NH_3, and heated at 90°C for 6 hours. After cooling, the compound **20** (0.26 g, 95%) obtained on evaporated of the solvent in vacuo, and then desilylated according to the same procedure described for preparation of **23** to give **26** (0.10 g, 75%) as white micro needles, recrystallized from MeOH: UV (H_2O) λ_{max} 279.0 nm (ε 8,040) (pH 7), 290.0 (ε 7,070) (pH2), 278.8 (ε 7,580) (pH11). Anal. ($C_9H_{12}N_6O_3$) C, H, N.

(-)-(2R,4R)-2-Amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine can be prepared by reduction of compound **21** using a variety of reducing agents, including palladium on carbon and hydrogen gas or tributyltin hydride and azabisisobutyronitrile.

II. Anti-HIV Activity of Dioxolane Nucleosides

[0057] β-D-Dioxolane-nucleosides can be used as research tools to inhibit the growth of HIV in vitro, or can be administered to humans pharmaceutically to inhibit the growth of HIV in vivo.

[0058] The ability of β-D-dioxolane-nucleosides to inhibit HIV can be measured by various experimental techniques. The technique used herein, and described in detail below, measures the inhibition of viral replication in phytohemagglutinin (PHA) stimulated human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV). The amount of virus produced is determined by measuring the virus-coded reverse transcriptase enzyme. The amount of enzyme produced is compared to an HIV control. The method is described in detail below.

Antiviral and Cytotoxic Assay in Human Peripheral Blood Mononuclear Cells.

[0059]

A. Three-day-old phytohemagglutinin-stimulated PBM cells (10^6 cells/ml) from hepatitis B virus and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100 times the 50% tissue culture infectious dose ($TICD_{50}$) per ml and cultured in the presence and absence of various concentrations of antiviral compounds.

B. Approximately 45 minutes after infection, the medium, with the compound to be tested (2 times the final concentration in medium) or without compound, was added to the flasks (5ml; final volume 10 ml). AZT was used as a positive control.

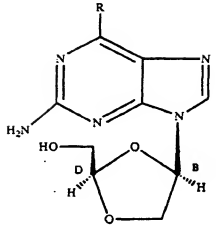
C. The cells were exposed to the virus (about 2 x 10^5 dpm/ml, as determined by reverse transcriptase assay) and then placed in a CO_2 incubator. HIV-1 (strain LAV) was obtained from the Center for Disease Control, Atlanta, Georgia. The methods used for culturing the PBM cells, harvesting the virus and determining the reverse transcriptase activity were those described by McDougal et al. (J. Immun. Meth. 76, 171-183, 1985) and Spira et al. (J. Clin. Meth. 25, 97-99, 1987), except that fungizone was not included in the medium (see Schinazi, et al., Antimicrob. Agents Chemother. 32, 1784-1787 (1988)). The reverse transcriptase activity in the virus-infected control was about 2 x 10^5 dpm per ml. Blank and uninfected cell control values were about 300 and 1,000 dpm, respectively. Similar results are obtained when Step C is performed before step B.

D. On day 6, the cells and supernatant were transferred to a 15 ml tube and centrifuged at about 900 g for 10 minutes. Five ml of supernatant were removed and the virus was concentrated by centrifugation at 40,000 rpm for 30 minutes (Beckman 70.1 Ti rotor). The solubilized virus pellet was processed for determination of the levels of reverse transcriptase. Results are expressed in dpm/ml of sampled supernatant.

[0060] The percent inhibition of virus, as determined from measurements of reverse transcriptase, is plotted versus the micromolar concentration of compound. The EC_{50} is the concentration of compound at which there is a 50% inhibition of viral growth.

[0061] Using this assay, it has been discovered that a small number of β-D-dioxolanyl purine nucleosides are potent anti-HIV agents. Specifically, as indicated in Table 1, compounds **21**, **25**, and **26** exhibit a low effective median concentration, ranging from 0.027 to 0.69 μM.

Table 1



R	Anomer	EC_{50}*
Cl	ß-D	0.9
Cl	ß-L	13.4
NH_2	ß-D	0.7
OH	ß-D	0.03

* Mean of at least 2 assays, using different donor cells. Standard error estimated at plus or minus 10%.

[0062] In contrast to the previous report that β-D-(±)-dioxolane-thymine has low efficacy against HIV in ATH8 cells, the enantiomerically pure β form **11** exhibited a potent anti-HIV activity (EC_{50} = 0.3 μM). It was surprising to discover that enantiomerically pure β-D-(-)-dioxolane-T has significantly higher anti-HIV activity than the racemic mixture of the compound. This difference may be explained based on the rate of phosphorylation of **11** in these systems. As expected, the a-isomer **12** did not exhibit any significant anti-HIV activity. The EC_{50} of (-)-1-[(2β,4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine in PBM cells was measured as 0.2 μM.

III. Toxicity of Dioxolane Nucleosides

[0063] The toxicities of compounds 21, 25, and 26, were evaluated in uninfected human PBM cells, CEM cells (T-lymphoblastoid cell line obtained from ATCC, Rockville, MD) and Vero (African Green Monkey kidney) cells. The three compounds were not toxic in any of the cell lines at a concentration of 100 µM.

IV. Preparation of Pharmaceutical Compositions

[0064] Humans suffering from HIV infection can be treated by administering to the patient an effective amount of (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine; (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine; (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine; or (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine or a pharmaceutically acceptable derivative or salt thereof, optionally in a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

[0065] The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount without causing serious toxic effects in the patient treated.

[0066] A preferred dose of the active compound for all of the above-mentioned conditions will be in the range from about 1 to 60 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

[0067] The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50-1000 mg is usually convenient.

[0068] Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 µM, preferably about 1.0 to 10 µM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

[0069] The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

[0070] A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

[0071] The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

[0072] The active compound or pharmaceutically acceptable salt or derivative thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

[0073] The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including other nucleoside anti-HIV compounds.

[0074] Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,

glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0075] If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

[0076] In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

[0077] Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

V. Preparation of Phosphate Derivatives of β-D-Dioxolane-Nucleosides

[0078] Mono, di, and triphosphate derivative of β-D-dioxolane-nucleosides can be prepared as described below.

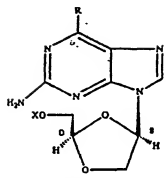
[0079] The monophosphate can be prepared according to the procedure of Imai et al., J. Org. Chem., 34(6), 1547-1550 (June 1969). For example, about 100 mg of β-D-dioxolane-nucleoside and about 280 µl of phosphoryl chloride are reacted with stirring in about 8 ml of dry ethyl acetate at about 0°C for about four hours. The reaction is quenched with ice. The aqueous phase is purified on an activated charcoal column, eluting with 5% ammonium hydroxide in a 1:1 mixture of ethanol and water. Evaporation of the eluant gives ammonium-(β-D-dioxolane-nucleoside)-5'-monophosphate.

[0080] The diphosphate can be prepared according to the procedure of Davisson et al., J. Org. Chem., 52(9), 1794-1801 (1987). β-D-Dioxolane-nucleosides can be prepared from the corresponding tosylate, that can be prepared, for example, by reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner (e.g., by washing, drying, and crystallizing it).

[0081] The triphosphate can be prepared according to the procedure of Hoard et al., J. Am. Chem. Soc., 87(8), 1785-1788 (1965). For example, β-D-dioxolane-nucleoside is activated (by making a imidazolide, according to methods known to those skilled in the art) and treating with tributyl ammonium pyrophosphate in DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate. Purification by anion exchange chromatography of a DEAE column is followed by isolation of the triphosphate, e.g., as the tetrasodium salt.

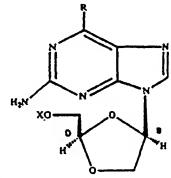
Claims

1. An enantiomerically pure B-D-dioxolanyl nucleoside of the structure:



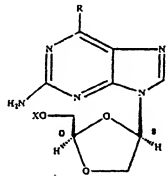
wherein R is NH_2, H or Cl and X is selected from the group consisting of hydrogen, acyl, monophosphate, diphosphate, and triphosphate, and wherein the compound is at least 97% free of the corresponding β-L enantiomer.

2. A pharmaceutical composition comprising an effective amount of an enantiomerically pure β-D-dioxolanyl nucleoside of the structure:



wherein R is NH_2, H or Cl and X is selected from the group consisting of hydrogen, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is at least 97% free of the corresponding β-L enantiomer, in a pharmaceutically acceptable carrier or diluent.

3. An enantiomerically pure β-D-dioxolanyl nucleoside of the structure:



wherein R is NH_2, H or Cl and X is selected from the group consisting of hydrogen, acyl, monophosphate, diphos-

phate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is at least 97% free of the corresponding β-L enantiomer, for use as a medicament.

4. Use of an enantiomerically pure β-D-dioxolanyl nucleoside as defined in any one of the preceding claims in the manufacture of a medicament for treating HIV infection in a patient.

5. A nucleoside as defined in claim 1 or claim 3, wherein R is NH_2.

6. A composition as defined in claim 2, wherein R is NH_2.

7. Use as defined in claim 4, wherein R is NH_2.

8. A nucleoside as defined in claim 1 or claim 3, wherein R is H.

9. A composition as defined in claim 2, wherein R is H.

10. Use as defined in claim 4, wherein R is H.

11. A nucleoside as defined in claim 1 or claim 3, wherein R is Cl.

12. A composition as defined in claim 2, wherein R is Cl.

13. Use as defined in claim 4, wherein R is Cl.

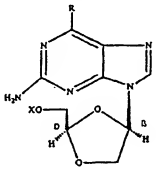
14. A nucleoside as defined in claim 5, claim 8 or claim 11, wherein X is H.

15. A composition as defined in claim 6, claim 9 or claim 12, wherein X is H.

16. Use as defined in claim 7, claim 10 or claim 13, wherein X is H.

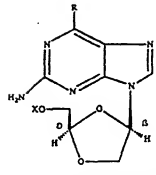
Patentansprüche

1. Enantiomerenreines β-D-Dioxolanylnukleosid der Struktur:



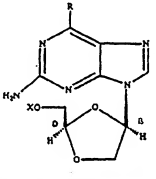
worin R NH_2, H oder Cl ist und X aus der Gruppe, bestehend aus Wasserstoff, Acyl, Monophosphat, Diphosphat und Triphosphat, ausgewählt ist und wobei die Verbindung zu mindestens 97 % frei von dem entsprechenden β-L-Enantiomer ist.

2. Pharmazeutische Zusammensetzung, umfassend eine wirksame Menge eines enantiomerenreinen β-D-Dioxolanylnukleosids der Struktur



worin R NH_2, H oder Cl ist und X aus der Gruppe, bestehend aus Wasserstoff, Acyl, Monophosphat, Diphosphat und Triphosphat, ausgewählt ist oder seines pharmazeutisch verträglichen Salzes, und wobei die Verbindung zu mindestens 97 % frei von dem entsprechenden β-L-Enantiomer ist, in einem pharmazeutisch verträglichen Träger oder Verdünnungsmittel.

3. Enantiomerenreines β-D-Dioxolanylnukleosid der Struktur:



worin R NH_2, H oder Cl ist und X aus der Gruppe, bestehend aus Wasserstoff, Acyl, Monophosphat, Diphosphat und Triphosphat, ausgewählt ist oder sein pharmazeutisch verträgliches Salz und wobei die Verbindung zu mindestens 97 % frei von dem entsprechenden β-L-Enantiomer ist, zur Verwendung als Arzneimittel.

4. Verwendung eines enantiomerenreinen β-D-Dioxolanylnukleosids nach einem der vorangehenden Ansprüche bei der Herstellung eines Arzneimittels zur Behandlung von HIV-Infektion bei einem Patienten.

5. Nukleosid nach Anspruch 1 oder Anspruch 3, wobei R NH_2 ist.

6. Zusammensetzung nach Anspruch 2, wobei R NH_2 ist.

7. Verwendung nach Anspruch 4, wobei R NH_2 ist.

8. Nukleosid nach Anspruch 1 oder Anspruch 3, wobei R H ist.

9. Zusammensetzung nach Anspruch 2, wobei R H ist.

10. Verwendung nach Anspruch 4, wobei R H ist.

11. Nukleosid nach Anspruch 1 oder 3, wobei R Cl ist.

12. Zusammensetzung nach Anspruch 2, wobei R Cl ist.

13. Verwendung nach Anspruch 4, wobei R Cl ist.

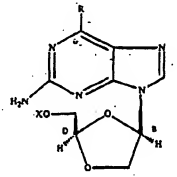
14. Nukleosid nach Anspruch 5, Anspruch 8 oder Anspruch 11, wobei X H ist.

15. Zusammensetzung nach Anspruch 6, Anspruch 9 oder Anspruch 12, wobei X H ist.

16. Verwendung nach Anspruch 7, Anspruch 10 oder Anspruch 13, wobei X H ist.

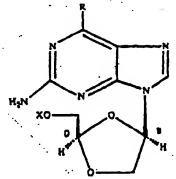
Revendications

1. Nucléoside de β-D-dioxolanyle énantiomériquement pur de structure



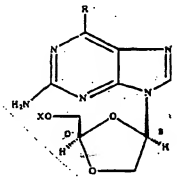
dans laquelle R est NH_2, H ou Cl, et X est choisi dans le groupe constitué de l'hydrogène, d'un groupe acyle, monophosphate, diphosphate et triphosphate, le composé étant au moins à 97% exempt de l'énantiomère β-L correspondant.

2. Composition pharmaceutique comprenant une quantité efficace d'un nucléoside de β-D-dioxolanyle énantiomériquement pur de structure :



dans laquelle R est NH_2, H ou Cl, et X est choisi dans le groupe constitué de l'hydrogène, d'un groupe acyle, monophosphate, diphosphate et triphosphate, ou de son sel pharmaceutiquement acceptable, le composé étant au moins à 97% exempt de l'énantiomère β-L correspondant, dans un véhicule ou un diluant pharmaceutiquement acceptable.

3. Nucléoside de β-D-dioxolanyle énantiomériquement pur de structure :



dans laquelle R est NH_2, H ou Cl, et X est choisi dans le groupe constitué de l'hydrogène, d'un groupe acyle, monophosphate, diphosphate et triphosphate, ou de son sel pharmaceutiquement acceptable, le composé étant au moins à 97% exempt de l'énantiomère β-L correspondant, pour un usage comme médicament.

4. Utilisation d'un nucléoside de β-D-dioxolanyle énantiomériquement pur selon l'une quelconque des revendications précédentes dans la fabrication d'un médicament pour le traitement d'une infection à VIH chez un patient.

5. Nucléoside selon la revendication 1 ou 3, dans lequel R est NH_2.

6. Composition selon la revendication 2, dans laquelle R est NH_2.

7. Utilisation selon la revendication 4, dans laquelle R est NH_2.

8. Nucléoside selon la revendication 1 ou 3, dans lequel R est H.

9. Composition selon la revendication 2, dans laquelle R est H.

10. Utilisation selon la revendication 4, dans laquelle R est H.

11. Nucléoside selon la revendication 1 ou 3, dans lequel R est Cl.

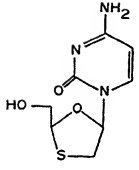
12. Composition selon la revendication 2, dans laquelle R est Cl.

13. Utilisation selon la revendication 4, dans laquelle R est Cl.

14. Nucléoside selon la revendication 5, 8 ou 11, dans lequel X est H.

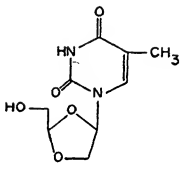
15. Composition selon la revendication 6, 9 ou 12, dans laquelle X est H.

16. Utilisation selon la revendication 7, 10 ou 13, dans laquelle X est H.



(±)-BCH-189

FIG. 1a



(±)-Dioxolane-T

FIG. 1b

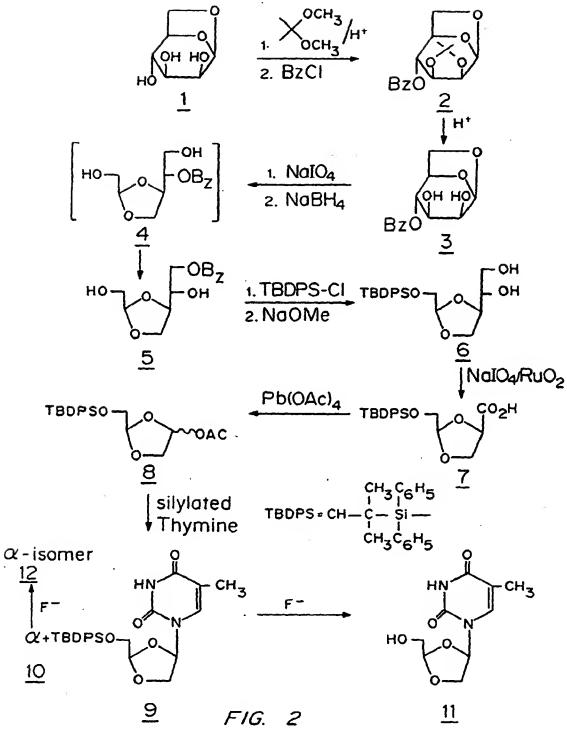
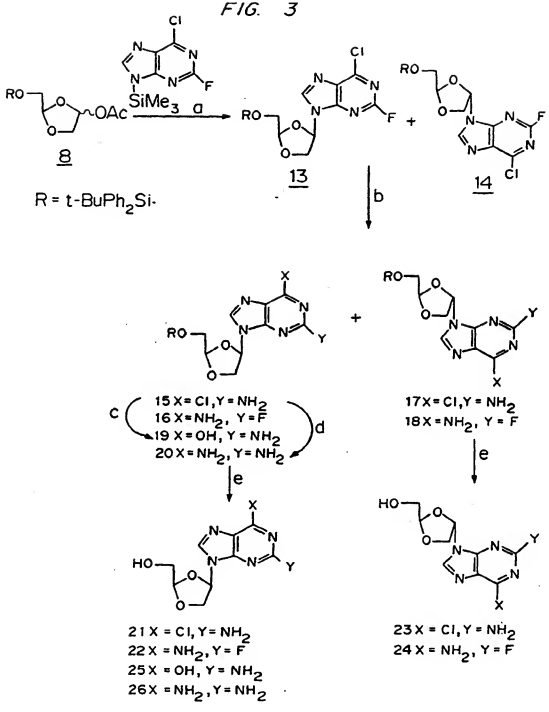

1. OCH3 OCH3 H+
2. BzCl
1
2
H+
3
1. NaIO4
2. NaBH4
4
1. TBDPS-Cl
2. NaOMe
5
6
NaIO4/RuO2
Pb(OAc)4
7
8
silylated Thymine
α-isomer
12
F−
α+TBDPSO
10
9
F−
11

FIG. 2

FIG. 3


RO
OAc
SiMe3
a
8
R = t-BuPh2Si-
13
14
b
15 X = Cl, Y = NH2
16 X = NH2, Y = F
19 X = OH, Y = NH2
20 X = NH2, Y = NH2
c
d
e
17 X = Cl, Y = NH2
18 X = NH2, Y = F
HO
21 X = Cl, Y = NH2
22 X = NH2, Y = F
25 X = OH, Y = NH2
26 X = NH2, Y = NH2
23 X = Cl, Y = NH2
24 X = NH2, Y = F